Supporting information for

A GluN2A-Selective Pyridopyrimidinone Series of NMDAR Positive Allosteric Modulators with an Improved *in vivo* Profile

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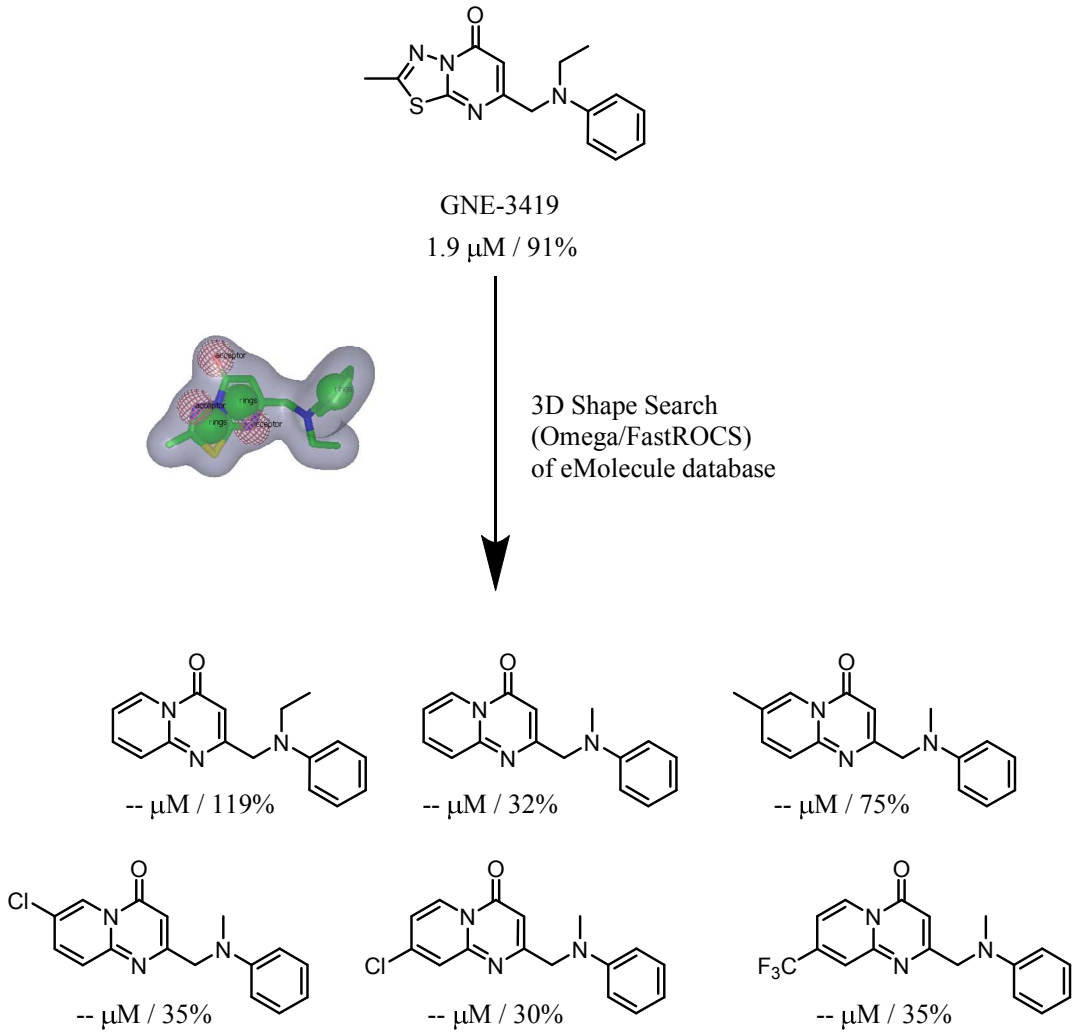
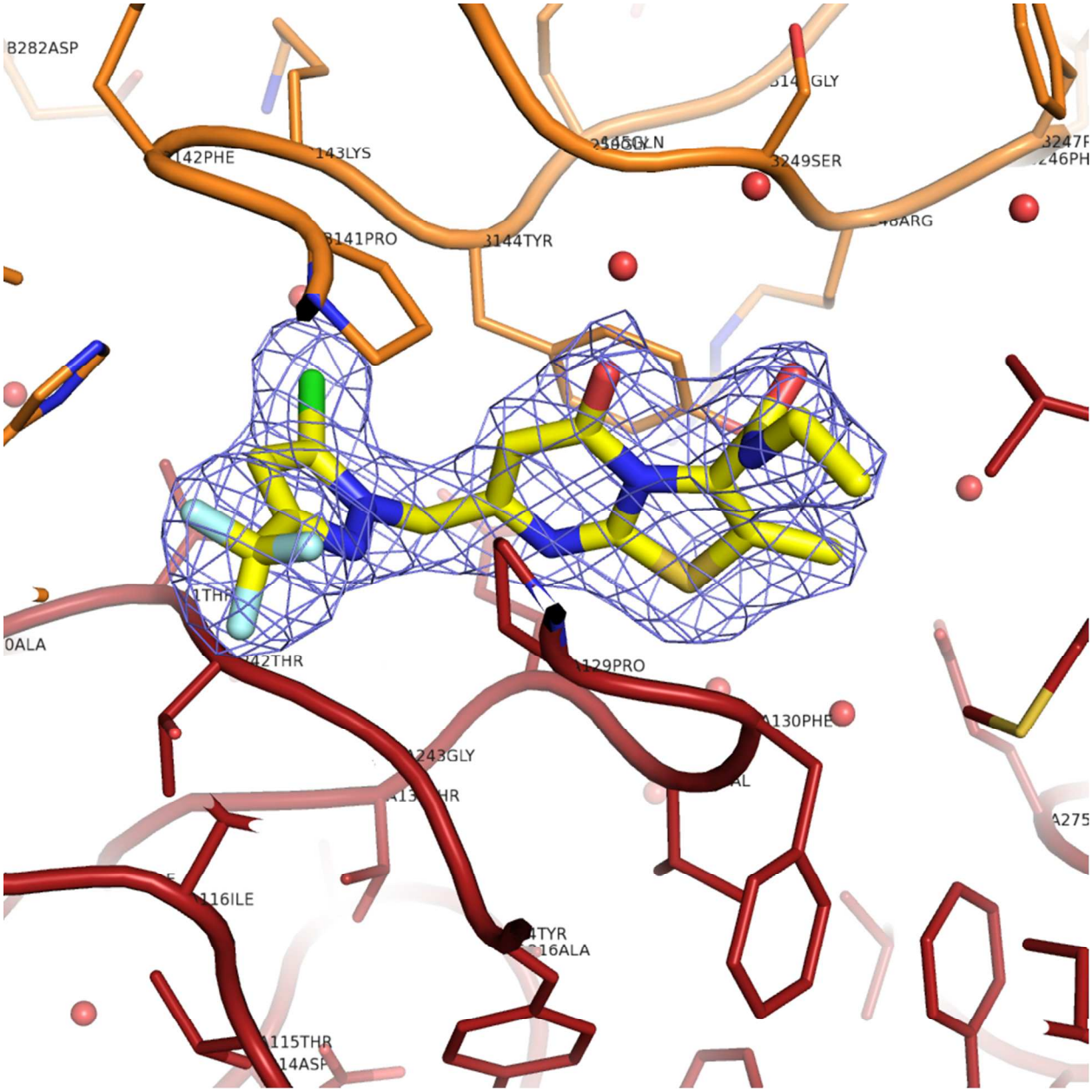
Figure S-1. 3D shape search identification of pyridopyrimidinone core



Pyridopyrimidinone compounds identified in a 3D shape search based on GNE-3419. A predicted 3D shape of GNE-3419 was used to search commercially available compounds from eMolecules. Shown are the pyridopyrimidinone-containing compounds that were ordered and the GluN2A EC_{50}'s and Maximum potentiation (EC_{50} of "—" means no curve fit was possible. 30% = no potentiation).

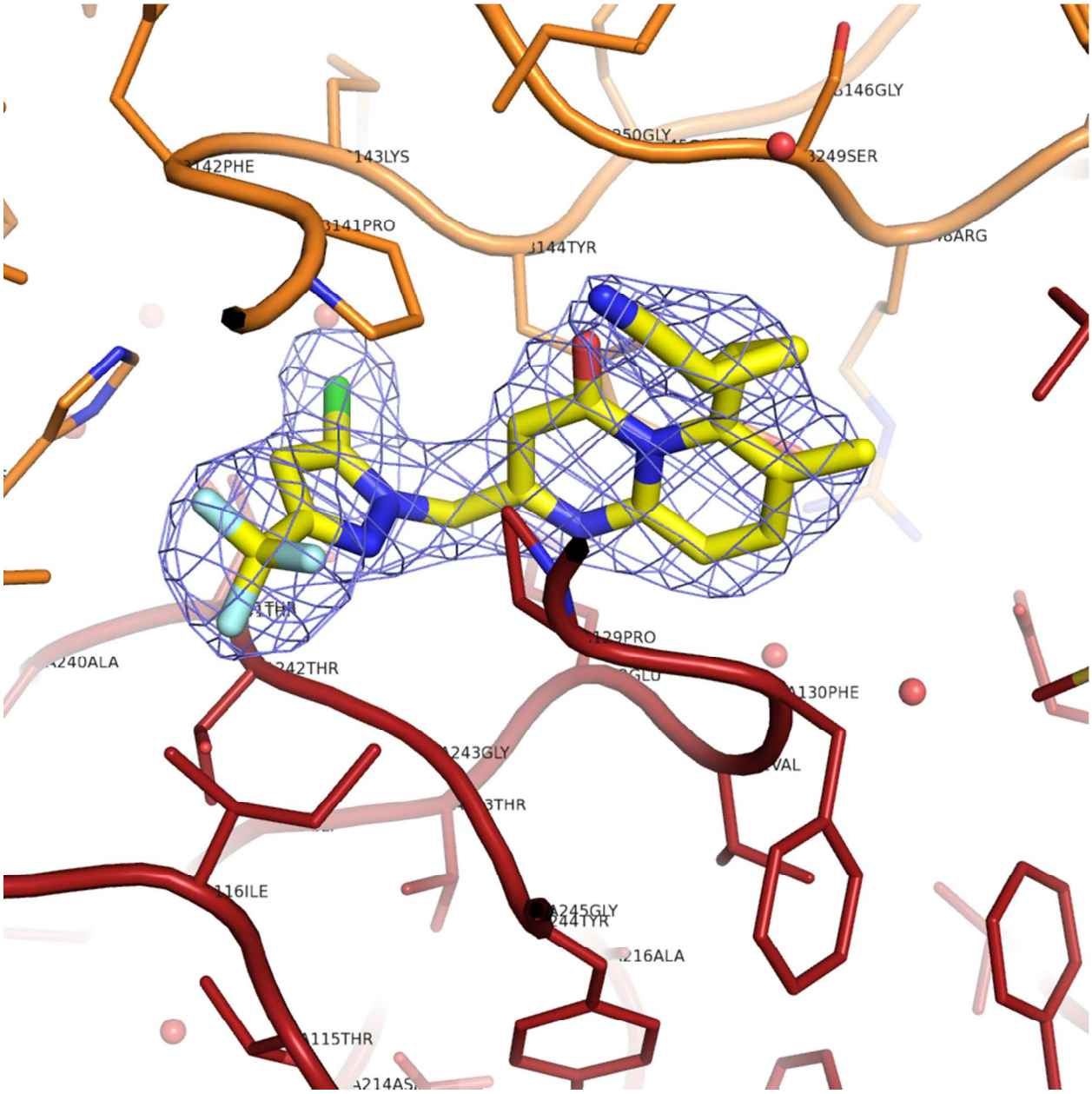
Figure S-2. Electron density map of Compound 2 bound to GluN1/GluN2A (PDB ID 5TP9)



X-ray co-crystal structure of compound **2** (yellow) bound to GluN1 (orange) and GluN2A (Red).

The electron density is shown at a contour level of 1 sigma.

Figure S-3. Electron density map of Compound 9 bound to GluN1/GluN2A (PDB ID 5TPA)



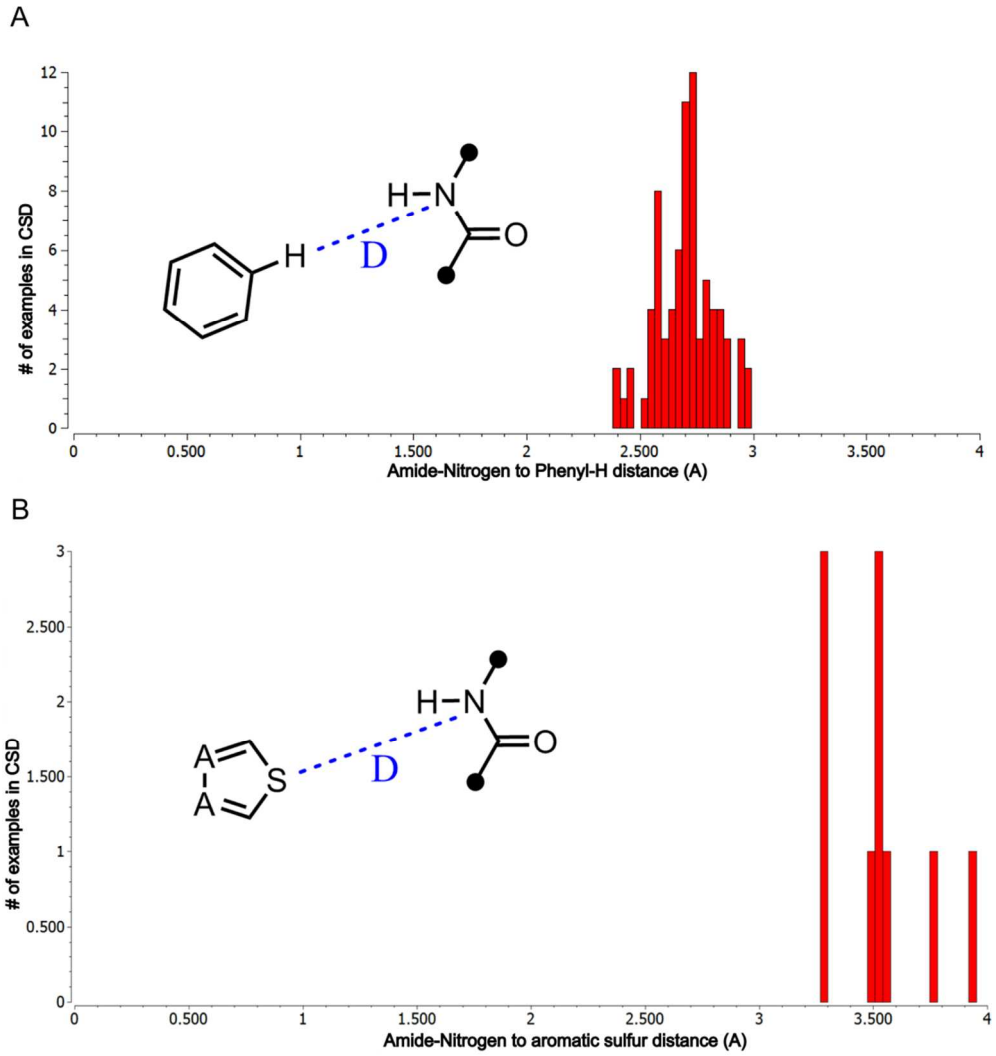
X-Ray co-crystal structure of compound 9 (yellow) bound to GluN1 (orange) and GluN2A (Red). The electron density is shown at a contour level of 1 sigma.

Table S-1. X-ray crystal structure data collection and refinement statistics

	GluN1/GluN2A-Cmpd **2** **PDB ID 5TP9**	GluN1/GluN2A-Cmpd **9** **PDB ID 5TPA**
Data collection	ALS 5.0.2	ALS 5.0.2
Space group	$P2_12_12_1$	$P2_12_12_1$
Cell dimensions		
a, *b*, *c* (Å)	56.26, 89.78, 120.86	55.76, 90.24, 122.70
α, β, γ (°)	90, 90, 90	90, 90, 90
Resolution (Å)	50–2.40 (2.49-2.40)*	122.66-2.48 (2.61–2.48) *
R_{sym} or R_{merge}	0.081 (0.0.722)	0.077 (0.763)
$I / \sigma I$	19.1 (2.7)	18.6 (2.5)
Completeness (%)	100 (100)	100 (100)
Redundancy	5.9 (6.1)	7.1 (7.3)
Refinement		
Resolution (Å)	47.67-2.39	72.69-2.48
No. reflections	24871	22620
R_{work} / R_{free}	19.2/23.7%	19.0/24.1%
No. atoms		
Protein	4398	4349
Ligand/ion	47	43
Water	124	147
Wilson B factor	55.2	62.3
Mean B factor	53.1	55.8
r.m.s. deviations		
Bond lengths (Å)	0.010	0.010
Bond angles (°)	1.08	1.14

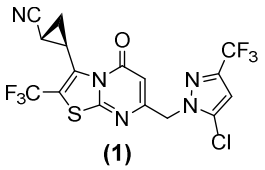
*Values in parentheses are for highest-resolution shell.

Figure S-4 Cambridge Structural Database distance statistics



A. Histogram of phenyl hydrogen-to-amide nitrogen distances with a edge-to-face interaction found in the Cambridge Structure Database (CSD). **B.** Histogram of thiophene sulfur to amide nitrogen distances with a edge-to-face interaction found in the Cambridge Structure Database (CSD).

Table S-2. Receptor Selectivity and DMPK Properties of compound 1

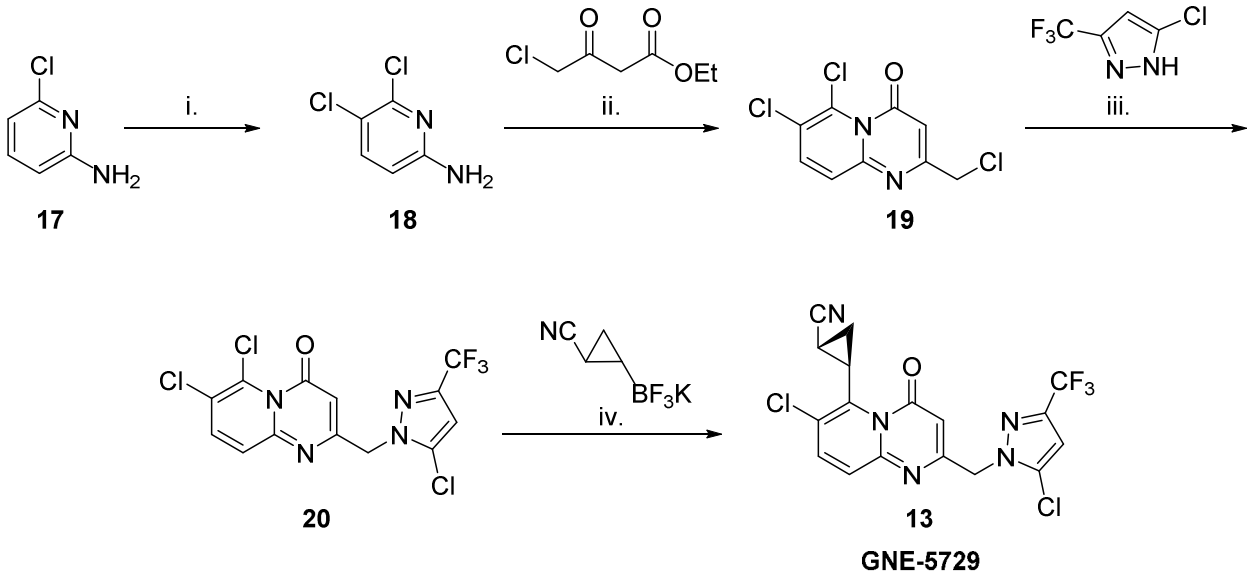


(1)

MW/LogD/TPSA		468 / 2.7 / 75	
NMDAR EC_{50} μM, (Max potentiation, %)[a] GluN2 A/B/C/D		**AMPAR EC_{50}, μM (Max potentiation, %)[b] GluA2 Flip/Flop**	
0.021 (152) / (49) / 7.4 (233) / 6.2 (160)		9.1 (84) / 5.5 (88)	
In Vitro DMPK			
LM[c] H/R/M[d] (mL/min/kg)	6 / 8 / 23	**PPB[i] (%) H/M[j]**	96.2 / 94
Hep[e] H/R/M[d] (mL/min/kg)	6 / 8 / 17	**Mouse Brain Binding (%)**	98.6
MDR1[f] ER[g] (B:A/A:B)[h]	2.1	**Kinetic Solubility (μM)**	9.3
Mouse PK			
IV Dosing (0.3 mg/kg)[k]		PO Dosing (10 mg/kg)[l]	
$Cl_{blood}/Cl_{blood,u}$ (mL min^{-1} kg^{-1})	26 / 433	**F (%)**	24
$t_{1/2}$ (h)	4	**$AUC_{last,u}$ (μM*h)**	0.20
V_{ss} (L/kg)	8.5	**$C_{max,u}$ (μM)[m]**	0.046
		$C_{brain,u}$ (μM)[n]	0.013
		$K_{p,uu}$[o]	0.62

[a]NMDAR EC_{50} values were determined in the presence of EC_{30} glutamate and saturating glycine. Max potentiation (%) at 125 μM reported if no EC_{50} could be obtained, where 30% denotes the assay baseline (EC_{30} glutamate). [b]AMPAR EC_{50} values were determined in the presence of 100 μM glutamate. Max potentiation (%) at 125 μM reported if no EC_{50} could be obtained, where 0% denotes the assay baseline due to receptor desensitization. All EC_{50} values represent geometric means of at least two determinations. [c]Liver microsome-predicted hepatic clearance. [d]H/R/M = human/rat/mouse. [e]In vitro stability in cryo preserved hepatocytes. [f]MDCK cells transfected with human MDR1 gene. [g]Efflux Ratio. [h]Basolateral-to-apical/apical-to-basolateral. [i]Plasma protein binding. [j]H/M = human/mouse. [k]Vehicle: 10% DMSO, 35% PEG400 in water. [l]Vehicle: MCT suspension [m]Free plasma concentration at C_{max}. [n]Free brain concentration at 1 h time point. [o]$K_{p,uu} = C_{brain,u} / C_{plasma,u}$ at 1 h time point.

Figure S-5. Synthesis of GNE-5729 (13)[a]



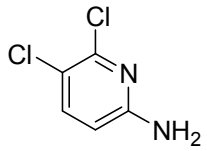
[a] Reagents and conditions: (i) NCS, CH_3CN, 80°C, 63% yield; (ii) PPA, 110°C, 31% yield; (iii) KI, K_2CO_3, CH_3CN, 80°C, 40% yield; (iv) $Pd(dppf)Cl_2$, K_3PO_4, 1,4-dioxane, H_2O, 90°C, 9% yield.

Procedure and Characterization

General Methods

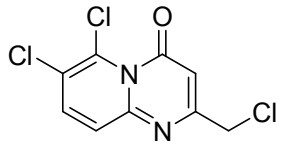
Unless otherwise indicated, all commercial reagents and anhydrous solvents were used without additional purification. 1H-NMR spectra were measured on Bruker Avance III 300, 400, or 500 MHz spectrometers. 13C-NMR spectra were measured on a Bruker Avance III 125.80 MHz spectrometer. Chemical shifts (in ppm) were referenced to internal standard tetramethylsilane (δ= 0 ppm). The reported carbon multiplicities and coupling constants are from C−F coupling. High-resolution mass spectrometry of final compounds was performed on a Thermo UHPLC/QE with a Thermo-Q Exactive mass spectrometry detector using ESI ionization, after elution on an Acquity BEH C18 (2.1 mm × 50 mm; 1.7 μm particle size) stationary phase using a gradient of water/acetonitrile (3−97% over 7 min; 0.1% formic acid in both phases). Reactions were monitored by walkup Shimadzu LCMS/UV system with LC-30AD solvent pump, 2020 MS, Sil-30AC autosampler, SPD-M30A UV detector, and CTO-20A column oven, using 2−98% acetonitrile/0.1% formic acid (or 0.01% ammonia) over 2.5 min, or by Waters Acquity LCMS system using 2−98% acetonitrile/0.1% formic acid (or 0.1% ammonia) over 2 min. Flash column chromatography purifications were done on a Teledyne Isco Combiflash Rf utilizing Silicycle HP columns. Reverse-phase purification was carried out on a Phenomenex Gemini-NX C18 (30 mm × 100 mm, 5um) with a gradient of 5−95% acetonitrile/water (with 0.1% formic acid or 0.1% NH4OH) over 10 min at 60 mL/min. Preparative SFC separations were performed on a PIC Solutions instrument, with conditions indicated in the Experimental Section. Analytical purity was greater than 95% as determined by LCMS using UV 254 nM detection unless stated otherwise. The melting point was determined by differential scanning calorimetry (DSC) (TA Instruments-Waters L.L.C.) by using 5 mg of solid sample and measuring the onset melting temperature.

Step 1: 5,6-dichloropyridin-2-amine (**17**)



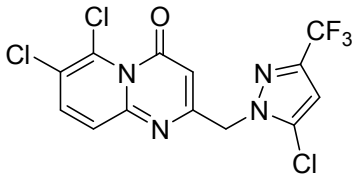
To a solution of 6-chloropyridin-2-amine (5.00 g, 38.9 mmol) in acetonitrile (50 ml) was added N-chlorosuccinimide (5.30 g, 39.3 mmol). The reaction was stirred for 18 h at 80 ^{o}C and then concentrated *in vacuo*. The residue was purified by chromatography with ethyl acetate/petroleum ether (1/3) to afford 5,6-dichloropyridin-2-amine (4.00 g, 63%) as a white solid. LCMS (ESI): $M+H^+$ = 163.0. ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.42 (d, J = 4.0 Hz, 1H), 6.63 (d, J = 4.0 Hz, 1H), 5.10 (brs, 2H).

Step 2: 6,7-Dichloro-2-(chloromethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (**18**)



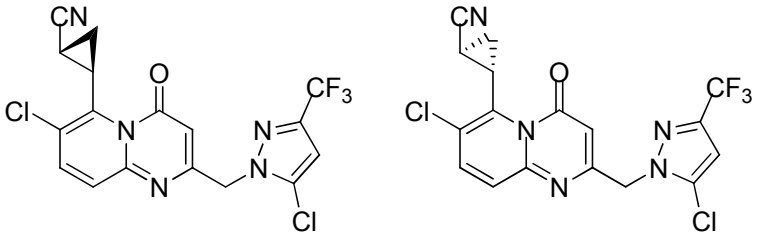
A mixture of 5,6-dichloropyridin-2-amine (4.00 g, 24.5 mmol), ethyl 4-chloro-3-oxobutanoate (8.10 g, 49.2 mmol) and PPA (21.0 g, 182 mmol) was stirred for 1 h at 110 ^{o}C. The reaction was poured into water (50 ml) and the pH value of the solution was adjusted to 7 with sodium hydroxide (1 mol/L). The resulting solution was extracted with dichloromethane (3x200 ml) and then concentrated *in vacuo*. The residue was purified by chromatography with ethyl acetate/petroleum ether (1/3) to afford 6,7-dichloro-2-(chloromethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (2.00 g, 31%) as a brown solid. LCMS (ESI): $M+H^+$ = 263.0. ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.58 (d, J = 4.8 Hz, 1H), 7.37 (d, J = 4.8 Hz, 1H), 6.57 (s, 1H), 4.45 (s, 2H).

Step 3: 6,7-Dichloro-2-[[5-chloro-3-(trifluoromethyl)-1H-pyrazol-1-yl]methyl]-4H-pyrido[1,2-a]pyrimidin-4-one (**19**)



To a solution of 6,7-dichloro-2-(chloromethyl)-4H-pyrido[1,2-a]pyrimidin-4-one (1.00 g, 3.80 mmol) in acetonitrile (50 mL) was added 5-chloro-3-(trifluoromethyl)-1H-pyrazole (519 mg, 3.04 mmol), potassium iodide (317 mg, 1.91 mmol) and potassium carbonate (1.05 g, 7.60 mmol). The reaction was stirred for 1 h at 80 ^{o}C. Then the resulting mixture was concentrated *inv acuo.* The residue was purified by chromatography with ethyl acetate/petroleum ether (1/9) to afford 6,7-dichloro-2-[[5-chloro-3-(trifluoromethyl)-1H-pyrazol-1-yl]methyl]-4H-pyrido[1,2-a]pyrimidin-4-one (600 mg, 40%) as yellow oil. LCMS (ESI): M+H^{+} = 397.1; ^{1}HNMR (300 MHz, $CDCl_3$) δ 7.60 (d, J = 4.8 Hz, 1H), 7.34 (d, J = 4.8 Hz, 1H), 6.60 (s, 1H), 5.85 (s, 1H), 5.31 (s, 2H).

Step 4: (1R,2R)-2-(7-chloro-2-[[5-chloro-3-(trifluoromethyl)-1H-pyrazol-1-yl]methyl]-4-oxo-4H-pyrido[1,2-a]pyrimidin-6-yl)cyclopropane-1-carbonitrile and (1S,2S)-2-(7-chloro-2-[[5-chloro-3-(trifluoromethyl)-1H-pyrazol-1-yl]methyl]-4-oxo-4H-pyrido[1,2-a]pyrimidin-6-yl)cyclopropane-1-carbonitrile (**13, GNE-5729**)



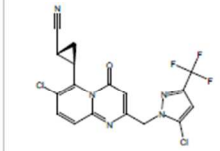
To a solution of 6,7-dichloro-2-[[5-chloro-3-(trifluoromethyl)-1H-pyrazol-1-yl]methyl]-4H-pyrido[1,2-a]pyrimidin-4-one (440 mg, 1.11 mmol) in 1,4-dioxane/H_2O (6 mL/0.6 mL) was added 2-[(1Z)-[(difluoropotassio)-lambda3-fluoranylidene]boranyl]cyclopropane-1-carbonitrile (577 mg, 3.34 mmol), 1,1'-Bis(diphenylphosphino)ferrocenepalladiumdichloride (250 mg, 0.342 mmol) and potassium phosphate (707 mg, 3.34 mmol). The resulting solution was stirred for 15 h at 90 ^{o}C and then concentrated *in vacuo.* The residue was purified with ethyl acetate/petroleum ether (1/9) to afford the racemic product (100 mg, 21%). Then this product was purified by Chiral-Prep-HPLC with the following conditions: Column, Chiralpak IC-3, 0.46*5cm, 3um;

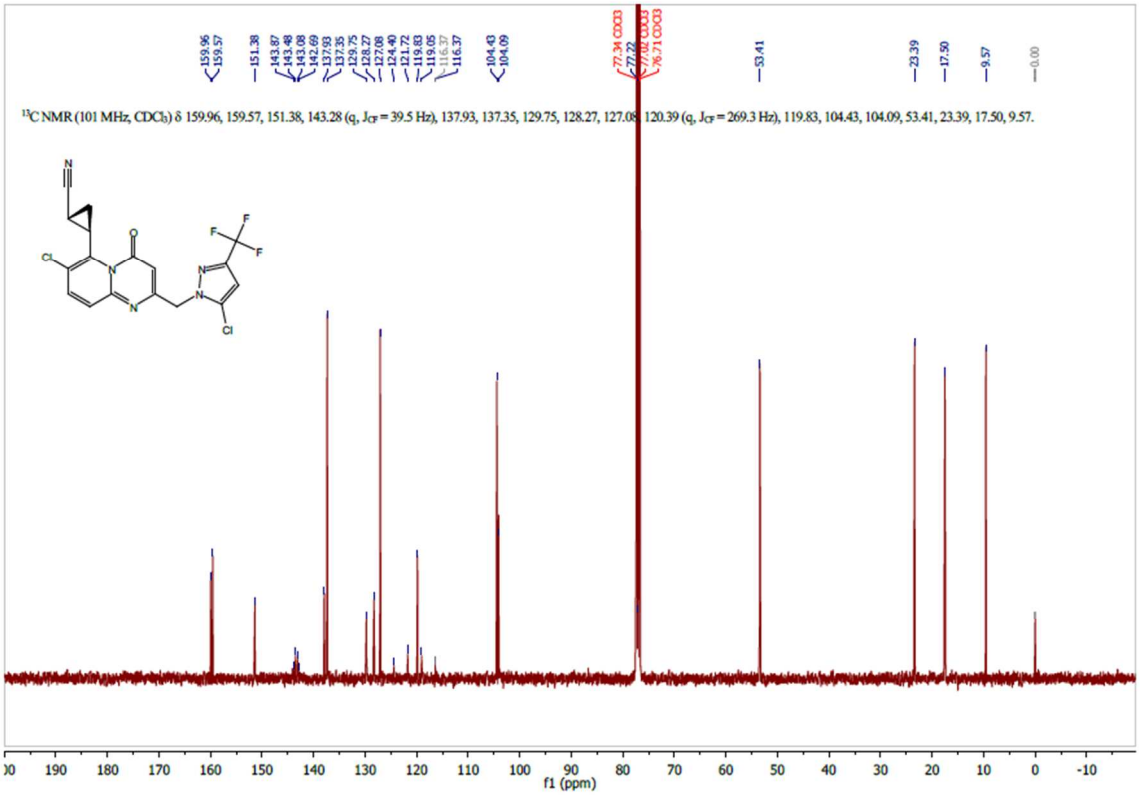
mobile phase, Hex and EtOH (hold 30.0% EtOH in 8 min); Detector, uv 254 nm to afford two isomers:

(Retention time, 4.082 min) GNE-5729 (**13**): (1R,2R)-2-(7-chloro-2-[[5-chloro-3-(trifluoromethyl)-1H-pyrazol-1-yl]methyl]-4-oxo-4H-pyrido[1,2-a]pyrimidin-6-yl)cyclopropane-1-carbonitrile as a yellow solid (40.7 mg, 9%). [α]D -134.56 (c. 0.3, in CH_2Cl_2); mp 136 °C (crystalline form), Tg 55.2 ^oC (amorphous form). HRMS (ESI) Calcd for $C_{17}H_{11}ON_5Cl_2F_3$ (M+H)+ = 428.0287. Found: 428.0273; ^1H NMR (400 MHz, $CDCl_3$) δ 7.50 (d, *J* = 9.5 Hz, 1H), 7.33 (dd, *J* = 9.5, 1.0 Hz, 1H), 6.61 (s, 1H), 5.86 (s, 1H), 5.33 (s, 2H), 3.33 – 3.23 (m, 1H), 1.84 (dt, *J* = 9.5, 6.0 Hz, 1H), 1.53 (dt, *J* = 9.1, 5.7 Hz, 1H), 1.21 (ddd, *J* = 9.2, 7.3, 6.1 Hz, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 159.96, 159.57, 151.38, 143.28 (q, J_{CF} = 39.5 Hz), 137.93, 137.35, 129.75, 128.27, 127.08, 120.39 (q, J_{CF} = 269.3 Hz), 119.83, 104.43, 104.09, 53.41, 23.39, 17.50, 9.57.

And (Retention time, 2.767 min) (1S,2S)-2-(7-chloro-2-[[5-chloro-3-(trifluoromethyl)-1H-pyrazol-1-yl]methyl]-4-oxo-4H-pyrido[1,2-a]pyrimidin-6-yl)cyclopropane-1-carbonitrile (42.3 mg, 9%) as a yellow solid. LCMS (ESI): $M+H^+$ = 428.0; ^{1}HNMR (300 MHz, CDCl3) 7.52 (d, J = 4.8 Hz, 1H), 7.37 (d, J = 4.8 Hz, 1H), 6.64 (s, 1H), 5.86 (s, 1H), 5.34 (s, 2H), 3.32-3.24 (m, 1H), 1.88-1.77 (m, 1H), 1.57-1.50 (m, 1H), 1.28-1.22 (m, 1H).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.50 (d, *J* = 9.5 Hz, 1H), 7.33 (dd, *J* = 9.5, 1.0 Hz, 1H), 6.61 (s, 1H), 5.86 (s, 1H), 5.33 (s, 2H), 3.33 – 3.23 (m, 1H), 1.84 (dt, *J* = 9.5, 6.0 Hz, 1H), 1.53 (dt, *J* = 9.1, 5.7 Hz, 1H), 1.21 (ddd, *J* = 9.2, 7.3, 6.1 Hz, 1H).





^{13}C NMR (101 MHz, $CDCl_3$) δ 159.96, 159.57, 151.38, 143.28 (q, J_{CF} = 39.5 Hz), 137.93, 137.35, 129.75, 128.27, 127.08, 120.39 (q, J_{CF} = 269.3 Hz), 119.83, 104.43, 104.09, 53.41, 23.39, 17.50, 9.57.